SUPPLEMENTAL MATERIAL

Methods

hECT Tissue Construction

hECTs were created from differentiated human embryonic stem cells (H7 cell line, NIH Registration #0061) and type-I collagen using methods previously described.[1,2]

hECT Functional Test Metrics

hECT twitch force and dynamics were assessed; hECT developed force (DF) was measured as the difference between maximum systolic and minimum diastolic force, using beam bending theory as described elsewhere.[3] Beat rate was measured from a sequence of contractions in spontaneously beating hECTs using previously established methods.[4]

hACF/HFF Conditioned Media

Conditioned media was collected from 10 cm dishes of 50% confluent hACFs or HFFs cultured in serum-free defined media (SFDM) for five days and used within 3 hours of collection.

Functional Assessment of hECTs Treated with hACF/HFF Conditioned Media

Following 48 hours of tissue compaction, hECTs were cultured in SFDM until day 5. Following spontaneous and 0.5 Hz pacing baseline contractile function testing on day 5, SFDM was replaced with the following treatments: 1) SFDM (Control); 2) fresh hACF conditioned media; or 3) fresh HFF conditioned media. hECTs were cultured an additional 5 days in their respective treatments, after which contractile function was measured and compared to baseline measurements. Spontaneous beat rate and DF were also measured on a daily basis.

hECT Immunofluorescence:

hECTs were fixed in 4% paraformaldehyde, and were later frozen and embedded in Tissue-Tek optimal cutting temperature compound (Sakura, Torrance, CA, USA). 10 μm sections were stained using either anti-cardiac troponin I (H-170, 1:100; Santa Cruz Biotechnology, Dallas, TX, USA) or anti-sarcoendoplasmic reticulum Ca^{2+}-ATPase 2 (MA3-919, 1:100; Invitrogen, Carlsbad, CA, USA), followed by Alexa Fluor 488 secondary antibody (A-11034, 1:200; Invitrogen) or Alexa Fluor 594 secondary antibody (A-11032, 1:200; Invitrogen), respectively. All sections were counterstained with 4',6-diamidino-2-phenylindole (DAPI); images were obtained using a laser-scanning confocal microscope (Leica TCS SP5 DMI; Leica Microsystems, Buffalo Grove, IL, USA) using a 63x oil immersion objective.

qRT-PCR

Total RNA was extracted from flash-frozen hECTs using the Quick-RNA MiniPrep kit (Zymo Research, Irvine, CA) after lysis using FastPrep Lysis Beads and Matrix Tubes (MP Products, Santa Ana, CA) and quantified using a NanoDrop 2000 (ThermoFisher, Rockville, MD). Reverse transcription was performed using the iScript cDNA synthesis kit (Biorad Laboratories, Hercules, CA) and quantitative PCR (10 ng cDNA/reaction) was performed using a two-step system with SYBR Advantage qPCR Premix (Clontech Laboratories, Mountain View, CA) on the ABI Prism 7500 Fast Real-Time PCR system (Applied Biosystems, Foster City, CA) according to manufacturer recommendations.

All primers used were human-specific and are shown in the 5' to 3' direction:

Gene	Direction	Sequence (5'→3')
β2M	Forward	GTATGCCTGCCGTGTGAAC
β2M	Reverse	CAAGCAAGCAGAATTTGGAA
TNNT2	Forward	AAGAGGCAGACTGAGCGGGAAA
TNNT2	Reverse	AGATGCTCTGCCACAGCTCCTT
αMYHC	Forward	GGAAGACAAGGTCAACAGCCTG
αMYHC	Reverse	TCCAGTTTCCGCTTTGCTCGCT
βMYHC	Forward	GGAGTTCACACGCCTCAAAGAG
βMYHC	Reverse	TCCTCAGCATCTGCCAGGTTGT
SERCA2a	Forward	CTGTCCATGTCACTCCACTTCC
SERCA2a	Reverse	AGCGGTTACTCCAGTATTGCAG
CASP3	Forward	TTAATAAAGGTATCCATGGAGAACACT
CASP3	Reverse	TTAGTGATAAAAATAGAGTTCTTTTGTGAG
CASP9	Forward	TTCCCAGGTTTTGTTTCCTG
CASP9	Reverse	CCTTTCACCGAAACAGCATT
BCL2	Forward	CTGCACCTGACGCCCTTCACC
BCL2	Reverse	CACATGACCCCACCGAACTCAAAGA
BAX	Forward	GGACGAACTGGACAGTAACATGG
BAX	Reverse	GCAAAGTAGAAAAGGGCGACAAC
LTCC	Forward	TGACTATTTTTGCCAATTGTGTGG
LTCC	Reverse	GCGGAGGTAGGCATTGGG
Cx43	Forward	GGGTTAAGGGAAAGAGCGACC
Cx43	Reverse	CCCCATTCGATTTTGTTCTGC
Kv4.2	Forward	CCAACTTCAGTCGCATCTACCAC
Kv4.2	Reverse	GCTCTGCATGTAAGCATTTGCGC
ANF	Forward	ACAATGCCGTGTCCAACGCAGA
ANF	Reverse	CTTCATTCGGCTCACTGAGCAC
Kir2.1	Forward	AACAGTGCAGGAGCCGCTTTGT
Kir2.1	Reverse	AGGACGAAAGCCAGGCAGAAGA
Kv11.1	Forward	CATCTGCGTCATGCTCATTGGC
Kv11.1	Reverse	TCTGGTGGAAGCGGATGAACTC
Nav1.5	Forward	CAAGACCTGCTACCACATCGTG
Nav1.5	Reverse	GTCGGCATACTCAAGCAGAACC

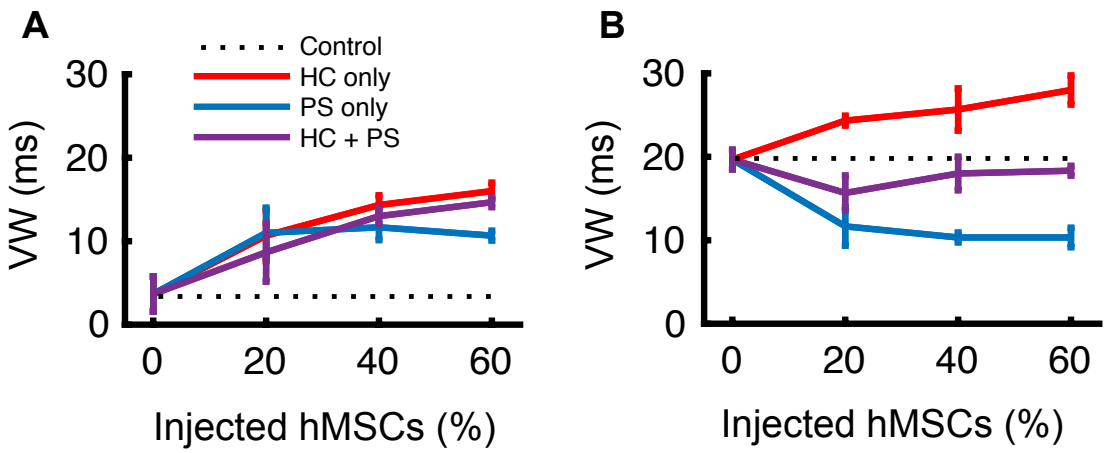
Fold changes in gene expression were determined using the comparative threshold cycle method (ΔΔCt) with normalization to the housekeeping gene beta-2-microglobulin (β2M). This experiment was repeated in triplicate.

Statistical Testing and Analysis

The descriptive statistics used to present results are specified within each figure legend. Statistical analyses were performed using Prism 6. Repeated measures analysis of variance (ANOVA), followed by Bonferroni's multiple comparisons test, was used for Figure 4. One-way ANOVA, followed by Scheffe's post-hoc test, was used for multiple pairwise comparisons of unequal group sizes in Figure 5. Differences with a p-value less than 0.05 were considered statistically significant.

Supporting References:

1. Turnbull IC, Karakikes I, Serrao GW, Backeris P, Lee JJ, Xie C, Senyei G, Gordon RE, Li RA, Akar FG, Hajjar RJ, Hulot JS, Costa KD. Advancing functional engineered cardiac tissues toward a preclinical model of human myocardium. *FASEB J*. 2014;28:644-654
2. Cashman TJ, Josowitz R, Gelb BD, Li RA, Dubois NC, Costa KD. Construction of defined human engineered cardiac tissues to study mechanisms of cardiac cell therapy. *J Vis Exp*. 2016:e53447
3. Serrao GW, Turnbull IC, Ancukiewicz D, Kim DE, Kao E, Cashman TJ, Hadri L, Hajjar RJ, Costa KD. Myocyte-depleted engineered cardiac tissues support therapeutic potential of mesenchymal stem cells. *Tissue Eng Part A*. 2012;18:1322-1333
4. Cashman TJ, Josowitz R, Johnson BV, Gelb BD, Costa KD. Human engineered cardiac tissues created using induced pluripotent stem cells reveal functional characteristics of braf-mediated hypertrophic cardiomyopathy. *PLoS One*. 2016;11:e0146697
5. Mayourian J, Cashman TJ, Ceholski DK, Johnson BV, Sachs D, Kaji DA, Sahoo S, Hare JM, Hajjar RJ, Sobie EA, Costa KD. Experimental and computational insight into human mesenchymal stem cell paracrine signaling and heterocellular coupling effects on cardiac contractility and arrhythmogenicity. *Circ Res*. 2017
6. White JW, Rassweiler A, Samhouri JF, Stier AC, White C. Ecologists should not use statistical significance tests to interpret simulation model results. *OIKOS Synthesising Ecology*. 2014;123:385-388



Online Figure I: Simulated Vulnerable Window (VW) Analysis on hMSC-Supplemented Fibrotic Cardiac Tissue. A VW analysis was performed on low (4%; left panel) and high (40%; right panel) fibroblast-populated cardiac tissue injected with 0% (control), 20%, 40%, or 60% hMSCs at high (16%) engraftment levels (n=3 randomized cell distributions). In low fibrosis (i.e., more representative of myocyte monolayers in vitro), both paracrine signaling (PS) and heterocellular coupling (HC) increases the VW, while in high fibrosis (i.e., more similar to pre-clinical/clinical conditions for hMSC intervention), PS decreases the VW and overcomes potentially pro-arrhythmic HC. Panel B adapted from Mayourian et al.,[5] which also provides detailed methods for these in silico simulations. As recommended by White et al.[6], comparative statistics were not implemented, as p-values are dependent on sample size (which can be arbitrarily high in simulations).